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# Assessment and Characterization of Microbial Communities in Salt Affected Soils on Galveston Island

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# ASSESSMENT AND CHARACTERIZATION OF MICROBIAL COMMUNITIES IN SALT AFFECTED SOILS ON GALVESTON ISLAND

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ASSESSMENT AND CHARACTERIZATION OF MICROBIAL COMMUNITIES IN SALT AFFECTED SOILS ON GALVESTON ISLAND

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# ABSTRACT

After Hurricane Ike in 2008, Galveston Island was flooded with sea water that left the soil and groundwater of the island with elevated sodium concentrations. As part of a long-term study that aims to restore plant life to the island, various soil amelioration techniques are being evaluated. Samples from bedded and non-bedded plots treated with gypsum, mulch, or both were assessed for microbial populations. Samples collected in July and October 2016 were cultured on Tryptic Soy Agar (bacteria enumeration), Pseudomonas Agar, Actinomycete Agar, and Rose Bengal Agar (fungi enumeration). Bacteria populations ranged from 4.07 to 5.12 log CFU/gram and from 3.73 to 4.26, pseudomonads from 4.09 to 5.21 and from 3.76 to 4.28, actinomycetes from 4.14 to 5.22 and from 3.87 to 4.32, and fungi from 3.27 to 3.58 and from 3.09 to 3.71 in summer then fall, respectively. There were no consistent statistical differences in microbial populations among the treatments. Respiration measurements were also compared with no differences. Samples collected from control plots in January 2017 were cultured on Pseudomonas Agar amended with 0, 5, and 10 percent salt. No statistical differences were found. Sixteen isolates were characterized and preserved for future study. The study indicates no discernible effects on microbial populations in the soil from any of the soil amelioration techniques tested.

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# INTRODUCTION

Galveston Island, located in the Gulf of Mexico off the coast of Southern Texas, has long been considered one of North America's most vulnerable barrier islands. The island is three miles wide with its highest point a mere 8.2 meters above sea level (Center, n.d.). Coastal erosion has been a major concern since the City of Galveston was founded in the early 1800s and remains a problem today as the city population and economic activity of the Port of Galveston continue to grow. The island is home to more than 50,000 people as of the 2010 census and houses one of the busiest ports in the United States for commercial shipping as well as cruise lines (Bureau, n.d.).

After Hurricane Ike swept the island in 2008, nearly 80% of the live oak trees on the island were killed due to salt water damage. The trees were significant to the cultural, historical, and environmental integrity of the island. The hurricane also left behind abnormally elevated concentrations of sea salt in the soils and near surface groundwater, a problem compounded by the low elevation of the island. The salinated water in the groundwater as well as the salinated soils have prevented a reestablishment of the live oak trees, important to the aesthetics and cultural identity of the island.

Salinization is considered one of the foremost threats to plants and crops around the world because of its effects on numerous aspects of plant metabolism and growth (Yang, 2014). Plants in salt-stressed soils are not able to absorb as much water through their roots which can create water stress, nutrient imbalances and internal accumulation of sodium, chloride, and other ions. Many plants rely on soil microorganisms and rhizosphere microbes to aid in nitrogen fixation, nutrient cycling, and other processes that are essential to healthy plant growth and propagation (Usha, 2011). Consequently, drastic changes in physical and chemical attributes in soils may upset the delicate plant-microbe relationship. Sodium affected soils have smaller average size pore spaces which can inhibit water and gas flow in the root zone of plants. High levels of sodium ions present can affect the ability of the plant to uptake other necessary ions and diminishes the rate of nitrate absorption (Ogle and St. John, 2010).

Total microbial populations in rhizosphere soil have been found to be as much as ten to fifty-fold higher than in non-rhizosphere soil. Increases in rhizosphere microbial activity have also been linked to increased plant activity and plant health (Paul and Clark, 1996). Due to the heavy reliance of plants on soil microorganisms, an evaluation of soil microbes and soil health is necessary to determine how the soil microorganisms are being affected by salinization and how this could be affecting the island plants.

Once the overall populations and respiration activities of the soil microorganisms are assessed and their salt tolerance evaluated, the

recommendations for plant species or soil treatments that could be beneficial to the restoration efforts can be made with a greater degree of certainty. If the microorganisms present are more conducive to the growth of certain types of plants or can be used to increase the salt tolerance of these or other plants, this knowledge can influence which plant species or which treatments are used for the recovery of the island plant communities.

Though a full recovery of the live oak population may not be feasible in the near future, a recovery of plant life to return the aesthetic value of the shore is more reasonable. Soil amelioration treatments and simple, affordable remediation techniques such as raised planting beds and organic matter amendments may be effective in improving soil conditions for plant reestablishment. Ornamental plants known to be salt tolerant can also be useful to help maintain shoreline integrity without sacrificing the beauty for which the island is known. The remediation of vegetation and soil conditions also depends on robust microbial populations in the soil. A microbial population study is therefore necessary to fully understand the plant-soil-microbe interactions under elevated salt levels and what must be done to remediate the soil enough for the recovery of lost plant species.

As a part of a large scale, long term study, several different soil amelioration techniques such as organic pine bark mulch and gypsum amendments and salt tolerant plant species are being evaluated at Moody Gardens on Galveston Island. Organic amendments can help lower the pH of coastal soils and can also

improve the soil root interface which helps improve the establishment of plant roots. Organic matter also improves water infiltration which is a common problem in salt affected soils and a particular problem for plant roots. The calcium ions in gypsum promote the displacement and leaching of sodium ions from the root zones of plants. Live Oak (*Quercus virginiana*) was included in the study because of its previous aesthetic and cultural importance to the island and the desire to reestablish the species in the area. The salt tolerant hybrid *Taxodium* X 'TX406' (a cross between bald cypress and Montezuma cypress) was chosen for its known salt tolerance and vigor. The third species, *Hibiscus hamabo* was chosen because of the desire for an ornamental plant that also displayed a reasonable degree of salt tolerance.

Objectives of this study were as follows:

1. Evaluate effects of soil amelioration techniques (raised planting beds, gypsum and incorporated pine bark amendments) on soil bacteria, fungi, pseudomonad, and actinomycete populations.
2. Evaluate the effects of each treatment on soil respiration activity.
3. Isolate and characterize salt tolerant fluorescent pseudomonads and preserve isolates for further study.
4. Use gathered data to evaluate overall soil viability using microbial diversity and respiration activity as indicators of soil health.

# LITERATURE REVIEW

## Assessment of Microbial Community Response to Salt Stress

Soil salinity is one of the main factors contributing to crop loss and plant instability today. In highly salinized soils, osmotic potential is low and nutrient cycling can be reduced. Hydraulic conductivity is also lowered with increasing salt concentration and loss of water can cause stunted growth and low plant productivity (Sall et al., 2015). Rising sea levels make this a growing concern, especially in coastal areas. Poor soil management and climate change causing the loss of native vegetation compounded by natural disasters such as hurricanes threaten the coastlines of vulnerable barrier islands such as Galveston Island.

The port of Galveston and the entire Gulf of Mexico is economically important as well as being a significant habitat for migratory birds, commercial seafood, and housing numerous oil and natural gas service operations. Coastal erosion can have significant direct and indirect impacts on many of these while endangering the populations of the coastal cities themselves (Bertrand-Garcia et al., 2012). Restoration projects commonly use native plants species that are well adapted to local soil conditions to stabilize coastal sediments.

However, as sea levels rise or if soil sodium concentrations spike suddenly

after a natural disaster, native plants may be unable to adapt to the increased salinity. Bioaugmentation with plant growth promoting bacteria has shown promise as a way to stimulate native plant growth in coastal soils with high salt concentrations, but an assessment of the native microflora's response to salt stress is necessary before bioengineered microbes or introduced species are tested as inoculants (Bledsoe and Boopathy, 2016).

Many scientists have investigated the response of microorganisms to salt stress with mostly unvarying results. The response of microbial activity and biomass in rhizosphere and bulk soil to increasing salinity is a general decrease with increasing salinity. Microbial activity can be measured by determining population MPN's (Most Probable Numbers), measuring soil respiration, or enzyme activity. Soil salinity is often expressed as electrical conductivity, osmotic potential, sodium concentration, or sodium adsorption ratio (SAR value).

Plant growth in one study was found to be severely inhibited by increasing salt concentrations with dry root weight being 2 to 3-fold higher in non-saline soils than in soils with higher saline content (Elmajdoub et al., 2014). Cumulative soil respiration in the same study also showed marked decreases with increasing soil salinity. Microbial biomass decreased with increasing salinity as well but rhizosphere soil samples maintained higher microbial biomass than did bulk soil samples. The overall decrease in microbial biomass after the 20-day incubation period from the lowest salt concentration to the highest salt concentration was around 31% in rhizosphere soil and 45% in bulk soil.

Another study (Sall and Ndour, 2015) assessed microbial response to salinity stress in tropical sandy soils amended with native shrub residues or inorganic fertilizer. Untreated control soil, soil treated with shrub residue, and soil treated with inorganic fertilizer were collected and treated in replicate with three different sodium concentrations. Each sample was incubated at 28°C for seven days with soil respiration being measured daily as an assessment of microbial activity in the soil.

The pH and electrical conductivity of each sample for each treatment was measured along with dehydrogenase activity and nitrification potential. Microbial biomass was measured to determine microbial response to each soil treatment and each salt concentration. The total microbial biomass was significantly greater in the organically amended soil than in the control or inorganically fertilized soil. The highest salt concentration reduced the microbial biomass by around 50% in the control soil and 43% in the organically amended soil. There was also a decrease in cumulative respiration with increasing electrical conductivity suggesting that salinity has a negative effect on microbial activity. Soil salinity may inhibit organic matter decomposition which would decrease the amount of carbon substrate available for microbial decomposition and therefore decrease respiration rates.

Decreasing soil osmotic and matric potential can also be caused by low water and high salt content in soils. Globally, 100 million hectares of arable land are damaged by high salt concentrations, accounting for 130 million dollars of crop

loss annually in Australia alone. Low osmotic potential can also affect nitrogen cycling and amino acid uptake, among other nutrient cycling issues (Chowdhury et al., 2011). When different salt concentrations were tested in different types of sand and sandy loam soils overall microbial biomass decreased as salt concentration increased and increased salt concentration also caused significant changes to microbial community structure. Cumulative respiration on the last day of testing was similar in both soil types, though respiration was lower by 65% in the sand with added salt and 75% in the sandy loam compared with the untreated soils of the same types. Fungal biomass was the most sensitive to decreasing osmotic potential and as salt concentrations increased, fungal biomass decreased, especially in the sandy loam, causing disruptions to microbial community structures.

## Isolation and Characterization of Salt Tolerant Microorganisms

Evaluating different microorganisms' values as inoculants to stimulate plant growth or act as biofertilizer can be a constructive step in a coastal plant restoration effort. Coastal plants rely on soil microorganisms and bioaugmentation with native salt tolerant organisms isolated from the area can stimulate microbial communities and therefore the plants they rely on. *Azospirillum*, for example are free-living plant promoting bacteria that are capable

of nitrogen fixation, especially in grasses. Some strains can tolerate salt concentrations up to 300 μM and show promise as biofertilizer in rice paddies (Usha, 2011).

Metabolic diversity, ability to degrade complex molecules, spore formation, and ability to withstand changes in osmotic or matric potential all contribute to the greater salt tolerance of some species of microorganisms. *Halophiles* or "salt-loving" microorganisms can be found in saline soils, extreme saline environments, and oceans worldwide. Some of these organisms isolated from soils have been shown to be extremely salt tolerant and are generally from a select few stalwart genera such as the spore forming *Bacillus* and the even more salt tolerant *Halobacillus* (Orhan and Gulluce, 2015).

However, local soil conditions and the concentrations of ions such as chloride, sulfate, carbonate, and bicarbonate can cause substitutions in complexes with sodium that lower the pH of the soil, creating unfavorable conditions for certain types of microbial communities (Shi et al., 2012). High salt concentrations and alkaline conditions created when bicarbonate ions are substituted in sodium complexes in the soil create harsh conditions under which few plants can grow. Spore forming and halotolerant microorganisms such as *Bacillus* and *Halomonas* are likely to be found in these severe conditions which are common in the arid soils of northeast China, for example. Assessing the soil conditions, ion concentrations, and microbial communities present in the study area are all necessary to truly understand the local environment.

Some salt tolerant, plant growth promoting organisms have been isolated by different researchers in varying environments in different parts of the world. A bacterium confirmed by 16S rRNA analysis as *Bacillus licheniformis* was isolated in India by Sharma et al., 2015, and in Argentina by Salomon et al., 2014, and both isolates showed high salt tolerance and plant growth promotion qualities. Salt tolerant strains of *Pseudomonas* are also widely studied in the floodplains of India and are known for their abundance in rhizosphere soil. Rhizosphere microorganisms can form mutually beneficial relationships with plants, helping the plant with nutrient cycling and nitrogen fixation while using root exudates as a food source (Paul and Clark, 1996).

## Rhizosphere Microorganisms

Total microbial populations in rhizosphere soil have been found to be as much as ten to fifty-fold higher than in non-rhizosphere soil. Increases in rhizosphere microbial activity have also been linked to increased plant activity and plant health. Important nutrient cycling processes such as nitrogen fixation, the breakdown of organic matter, phosphorus uptake, and disease suppression via the out-competition of pathogenic organisms occur within the plant rhizosphere (Ingham, n.d.). Rhizosphere microorganisms in turn feed on the amino acids, sugars, and photosynthetic carbon released by the plant roots.

There are numerous incidents of plant growth-stimulating bacteria found to be associated with the rhizospheres of plants grown in salinated soil. In one study, isolates from rhizosphere soil that promoted seed germination and growth by at least 20% over the uninoculated control seeds were considered as plant growth-stimulating bacteria. These isolates were used to inoculate wheat seeds that were grown for four weeks to determine the inoculants' effects on root and plant growth. Eight of the original isolates were classified as plant growth promoting and were identified as *Bacillus, Pseudomonas, Staphylococcus, Enterobacter, Alcaligenes, Acinetobacter,* and *Pantoe.* These eight strains were also tested for salt tolerance with *Pseudomonas* showing significant growth even at the greatest salt concentration tested (Egamberdieva et al., 2008).

A fast-growing world population and therefore a growing demand for food has forced the use of more land for agriculture, including arid or coastal lands previously considered unusable. Modern irrigation and fertilization technologies make repurposing these lands for commercial agriculture more manageable, but some soils require proactive oversight (Damodaran and Jha, 2014). There has been some success in some such sodic soils or other similarly agriculturally unproductive land where using rhizosphere bacteria has induced salt tolerance in plants.

Sodic soils are classified as having a pH above 8.5, an exchangeable sodium percentage above 15, and high concentrations of free carbonates and bicarbonates. Hydraulic conductivity is generally poor and root impedance is a

major problem for most plants in these soils. Poor ion exchange, high salt concentrations, and water flow retardation not only affects plants, but also the rhizosphere organisms that rely on them. Salt tolerant rhizosphere organisms may be able to be used to improve the health of the soil to promote plant growth.

Sixteen rhizosphere and endophytic bacteria strains isolated from grasses showed plant promotion properties and were tested using rice seedlings with some bacteria strains increasing the percentage of germination to over ninety percent (Damodaran and Jha, 2014). The six strains with the highest plant growth promotion (rice seedlings with a high seedling vigor index and high percentage germinated) were characterized. All six strains were found to be six different species of the *Bacillus* genus. The gladiolus corms that were inoculated with these bacterial strains before planting and during the critical stages of growth showed the least symptoms of salt stress. The rhizosphere soil of these plants showed a significant decrease in the sodium adsorption ratios from before and after planting.

Rhizosphere organisms can also be instrumental in helping plants recover after a drastic change to environmental conditions or a sudden physiological stressor. Plant growth promoting rhizobacteria (PGPR) can induce their plant hosts to produce metabolites, and upon exposure to stress the plants can respond more readily and efficiently. Commonly studied PGPR genera such as *Bacillus* and *Pseudomonas* have been shown to improve the yields of tomato, peppers, and apples.

Other PGPRs produce defense compounds that fight plant pathogens. Grapevine plants produce terpene compounds that accumulate in leaf tissue and defend against pathogens and herbivore attacks. Although induction of the accumulation of these terpenes because of PGPRs has been poorly studied, (Salomon et al., 2014) successfully induced terpene synthesis in leaves and roots inoculated with *Bacillus licheniformis* and *Pseudomonas fluorescens*.

# MATERIALS AND METHODS

## Project Design

Forty-eight treatment plots, each measuring four meters by three meters located on Galveston Island, Texas near Scholes International Airport, west of Moody Gardens (Figure 1) were positioned in two rows of twenty-four plots each. The forty-eight plots were divided into six randomized replication blocks of eight treatments. Half of the plots within each block were bare ground (flat). The other half were raised beds, approximately 60 centimeters above the native soil made with nonnative bank sand (Jardina Soil, League City, Texas). Soils within each plot were amended with one of the following treatments: approximately eight centimeters of pine bark mulch incorporated to a depth of 15 cm, 5.1 kilograms of calcium sulfate incorporated to a depth of 15 cm, equivalent amounts of incorporated pine bark mulch and calcium sulfate, and controls with no amendments. Treatments were randomized within each block for a randomized complete block design. Each plot was planted with two each *Quercus virginiana* (live oak), *Hibiscus hamabo*, and *Taxodium* X 'T406' (the latter a cross between Bald cypress and Montezuma cypress introduced by the Nanjing Botanical Garden) (Figure 2). Slow release

fertilizer Osmocote® (63 grams per plant at the time of planting, and 30.5 grams per plant in May 2016) was applied in a 60 cm diameter circle around the root collar of each tree before the top mulch was applied. Plots were irrigated using both spray irrigation and drip irrigation systems that used reclaimed wastewater treated with reverse osmosis. Soil samples to test soil chemistry attributes were taken prior to soil treatments being applied and at designated times throughout the study. Soil sampling for this study began five months after planting was completed.

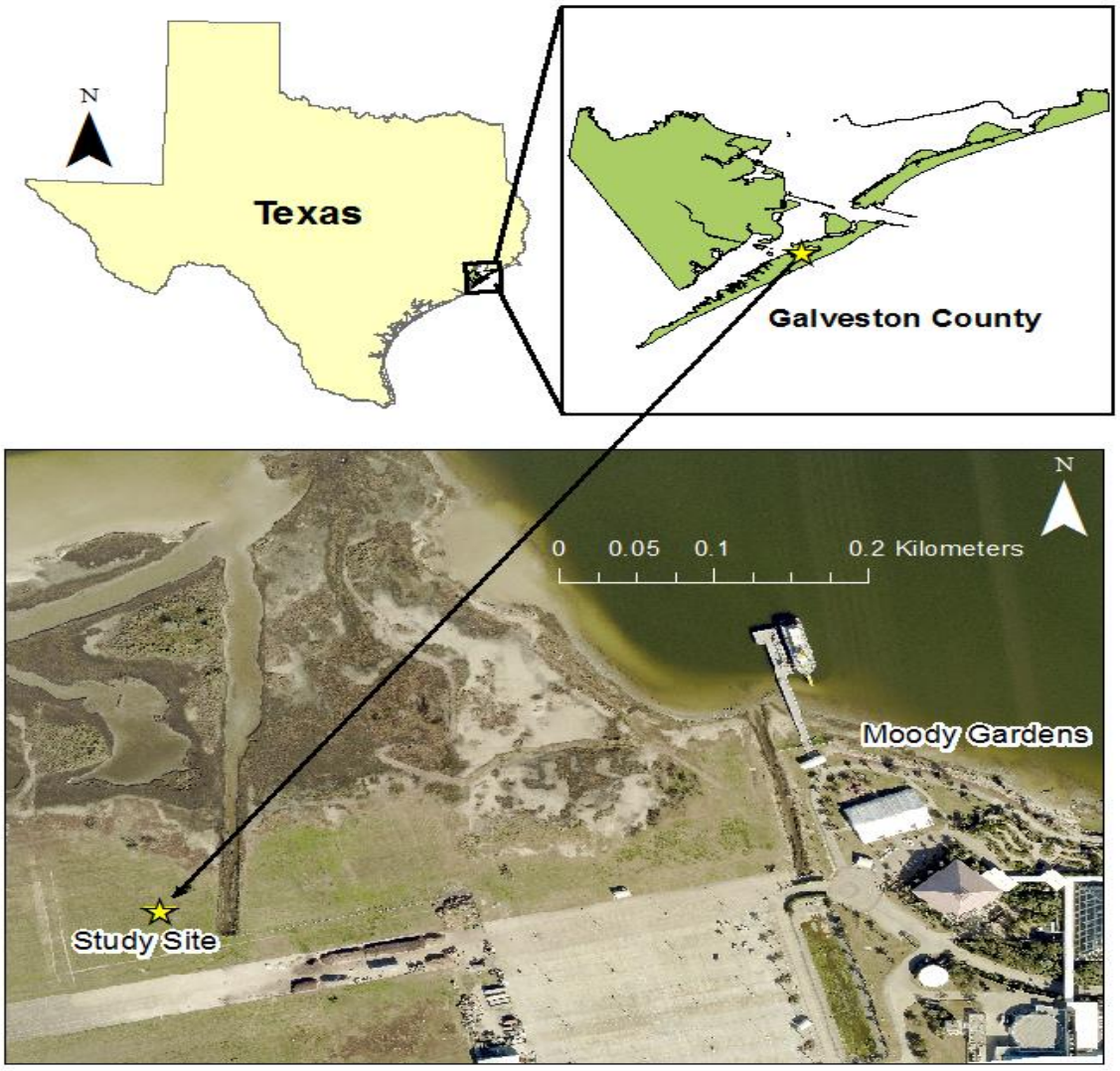



Figure 1. Location of Moody Gardens Study Site Near Scholes International Airport on Galveston Island, Texas.

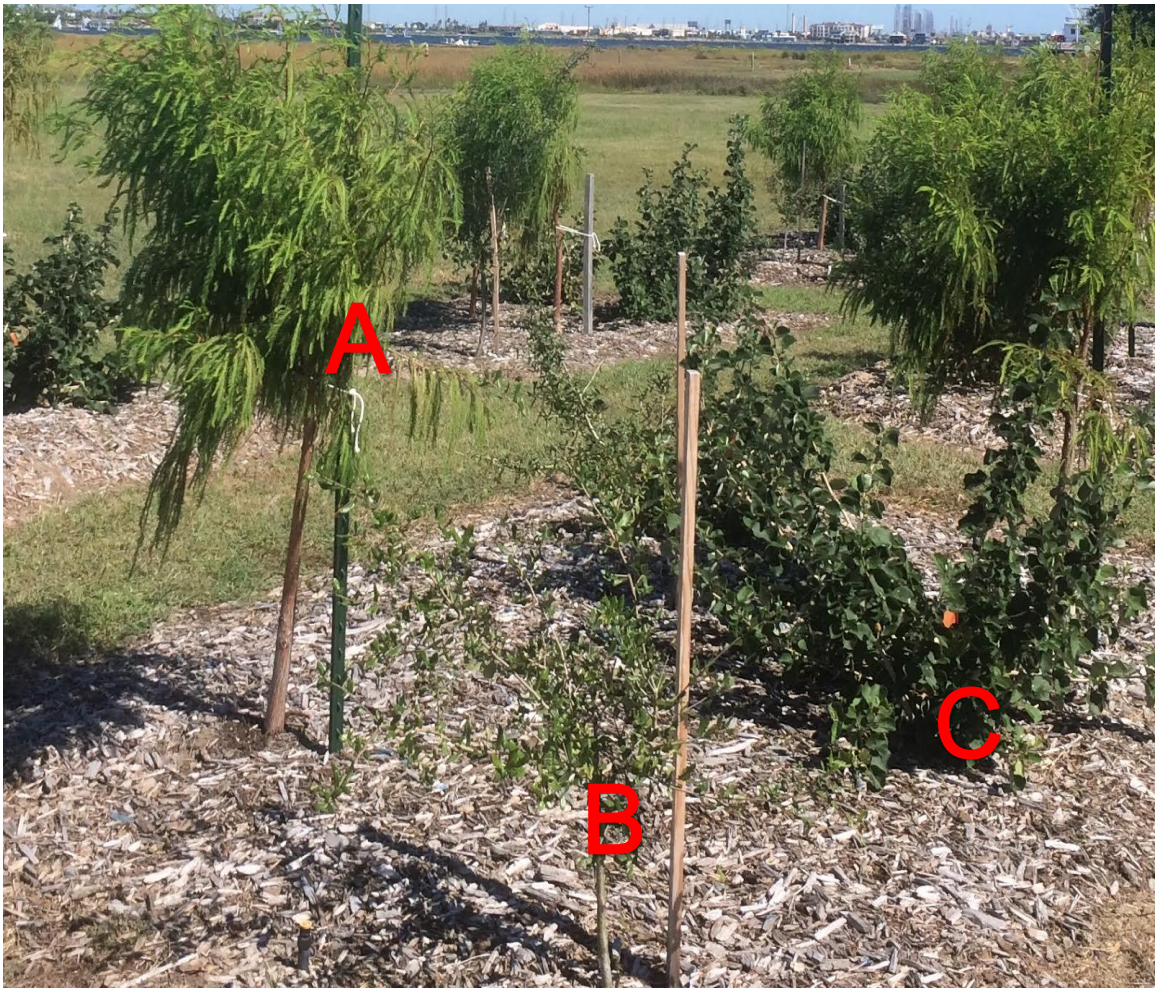


Figure 2. *Taxodium* X ‘T406’ (Montezuma/Bald Cypress hybrid, Tree A), *Quercus virginiana* (Live Oak, Tree B), and *Hibiscus hamabo* (Tree C), one year, three months after tree planting at the Moody Gardens test plots.

Table 1. Sample replication with eight randomized plots and treatment abbreviations used herein. This replication was repeated six times for forty-eight total plots and six total replications.

| Treatment | Abbreviation | Soil Type | Bed Type | Mulch | Gypsum |
|---|---|---|---|---|---|
| Control Bedded | CB | Nonnative | Raised | - | - |
| Control Flat | CF | Native | Flat | - | - |
| Mulch Flat | MF | Native | Flat | + | - |
| Mulch Bedded | MB | Nonnative | Raised | + | - |
| Gypsum Flat | GF | Native | Flat | - | + |
| Gypsum Bedded | GB | Nonnative | Raised | - | + |
| Mulch/Gypsum Bedded | MGB | Nonnative | Raised | + | + |
| Mulch/ Gypsum Flat | MGF | Native | Flat | + | + |

## Soil Sample Collection

Soil samples were collected from the research plots on July 19, 2016, October 22, 2016, and January 16, 2017. Samples were collected from all six replications of each treatment in July. After finding few significant differences among treatments, the sampling was reduced to four randomly selected replications collected in October. Samples were collected from control plots to test for the presence of salt tolerant pseudomonads in January 2017. Several randomly placed soil cores were taken from each plot to a depth of 15 cm using a push probe. Samples were stored in sealable plastic bags and placed in a cooler with ice for transport to the laboratory and were stored at 4°C until processed.

## Microbial Population Enumerations

Each sample was serially diluted to $10^{-3}$ with sterile phosphate buffer (Weber Scientific). In July the $10^{-2}$ and $10^{-3}$ solutions were used to inoculate Tryptic Soy Agar (TSA, Appendix B) for total bacteria enumeration, Pseudomonas Isolation Agar (PIA, Appendix C) for pseudomonad enumeration, Actinomycete Agar (ACT, Appendix D) for actinomycete enumeration, and Rose Bengal Agar (RBA, Appendix E) for total fungi enumeration in triplicate using a Model D automated spiral plater (Spiral Systems, Inc.). In October and January, only the $10^{-3}$ solutions were used to inoculate TSA, PIA, and ACT plates, and only the $10^{-2}$

solution was used to inoculate RBA plates. Plates were incubated at 25˚C and colonies counted twice to ensure that slow growing, fastidious organisms would be included in the population counts. TSA and PIA plates were counted after two and then four days. ACT plates were counted after three and then five days. RBA plates were counted after four and then seven days.

## Soil Respiration Determination

Soil temperature measurements were taken from three selected replications of the research plots using a digital temperature probe. Three soil respiration readings were taken in the field at random points within each of the selected plots using a Model EGM-4 Environmental Gas Monitor for $CO_2$ (PP Systems, 2010). The EGM-4 instrument creates a calibration line based on ambient $CO_2$ present while the sampling unit is held in the open air. This calibration reading was taken before sampling at every plot after turning off the sampling unit when traveling between plots. The sampling unit in recording mode was then placed directly on to the soil and the total change in concentration of $CO_2$ in the air drawn into the sampler from the soil was measured for 154 seconds or until the amount of $CO_2$ being measured surpassed ambient $CO_2$ levels. This change in concentration of $CO_2$ over time was plotted in real time during sampling using PP Systems Transfer Software© which automatically calculates the area under the

curve (total soil respiration in grams of $CO_2$ evolved per square meter of soil per hour) (*PP Systems Transfer Software*, 2009).

## Salt Tolerant Pseudomonad Population Assessment

Soil samples were collected from two replications of control flat and control bedded plots to collect and assess salt tolerant fluorescent pseudomonads. Samples were collected, stored, and processed as previously described. The $10^{-3}$ solution was used to inoculate PIA media amended with three salt concentrations (0, 5, and 10% w/v). Fluorescent colonies were counted using a handheld ultraviolet lamp to test for the presence of salt tolerant or halophilic fluorescent pseudomonads. Colonies suspected to be fluorescent pseudomonads were randomly selected from each treatment and the five and ten percent salt concentrations. These were preserved on nutrient agar slants for additional study.

## Characterization of Fluorescent Pseudomonads

Fluorescent *Pseudomonas* strains were randomly chosen from preserved enumeration plates, named, and transferred to a new plate using a sterile toothpick. After incubation at 25˚C for 24 hours, streak plates were made of each

organism on PIA with the same salt concentration from which they were originally isolated. The streak plates were incubated at 25˚C for 24 hours and the process was repeated. After 24 hours, single colony isolates from the second streak plates were subcultured to nutrient agar slants and plates, and nutrient broth stock cultures. The stock cultures were incubated at 25˚C for 24 hours. The slants and streak plates were stored at 4˚C for additional tests while the broths were used to create smear slides. The slides were heat fixed and Gram stained using the standard method (Brown and Smith, 2017).

Each fluorescent isolate was screened for the presence of the catalase enzyme using hydrogen peroxide and for production of cytochrome C oxidase using BBL™ DrySlides™ (Becton, Dickinson and Company). The isolates were also screened for glucose fermentation using phenol red broth (Becton, Dickinson and Company).

Single colony isolates from the preserved streak plates were transferred to tryptic soy agar plates and incubated for 24 hours at 25˚C. After incubation, single colony isolates from the TSA plates were transferred to TSA slants in screw cap tubes and plates amended with 5 percent salt. After incubation at 25˚C for 24 hours, the slants were stored in a 4˚C cooler. Growth from the plates was used to inoculates tryptic soy broth amended with 5 percent salt. After incubation for 24 hours at 25˚C, 500µL of tryptic soy broth was used to inoculate 20% glycerol stock solutions. The solutions were placed in a -80˚C freezer for long-term preservation.

## Gravimetric Moisture Content

The moisture content of each soil sample was determined. Ten grams of each soil were weighed into pre-weighed glass petri dishes. The soils were dried in an oven until a constant weight was achieved. The moisture content for each sample was calculated using the following equation:

$$\Theta_g = (M-D)/D$$

$\Theta_g$ = gravimetric moisture content
M = wet weight of sample
D = dry weight of sample

## Statistical Analysis

A single classification analysis of variance (ANOVA) was performed using SAS version 9.2 statistical software (*SAS*, 2017) comparing microbial populations between each treatment (CF, CB, MF, MB, GF, GB, MGF, MGB) for the first two sampling periods. An ANOVA was performed to test for differences among treatments for total bacteria, pseudomonads, fungi, and actinomycetes. A single classification ANOVA was performed on the soil respiration data and comparing fluorescent pseudomonads between the three salt concentrations (0, 5, 10%) tested.

# RESULTS AND DISCUSSION

## Soil Chemistry

Average pH measurements ranged from 8.28 to 8.61 before the application of the treatments and from 8.15 to 8.44 after the application of soil treatments. Electrical conductivity ranged from 624 µS/cm to 1983 µS/cm before treatments were applied, and from 981 µS/cm to 1328 µS/cm after. Sodium adsorption ratio values ranged from 0.99 to 2.81 before treatment application and from 1.30 to 2.31 after the treatments were applied. Sodium concentrations in the soil ranged from 83 parts per million (ppm) to 189 before treatments were applied and from 122 ppm to 209 after treatments were applied (Table 2). According to the USDA, sodium adsorption ratios between 0 and 12 and pH values below 8.5 classify salt affected soils as weakly saline, but not sodic (Scianna, 2002). Sodium concentrations remained relatively low in surface soils at the study site throughout the sampling periods. This may have been due to the combination irrigation system used and natural precipitation. The steady influx of water could have pushed sodium out of the surface soils. This is supported by the low (0%) mortality rate of the trees in the companion study indicating the plants were not being subjected to

significant salt stress in the soil. Overall sodium concentrations in the surface soil were not high enough to cause mortality to the plants. Soil samples taken at the beginning of the study at greater depths (some deep enough to contact the island's shallow water table) showed increasing salinity with increasing depth.

Table 2. Soil chemistry data for samples collected on February 19, 2016 before treatments were applied and 10/27/2016 after the treatments were applied.

| Treatment | Ec Before[1] | Ec After[2] | SAR Before[3] | SAR After[4] | Na Before[5] | Na After[6] | pH Before[7] | pH After[8] |
|---|---|---|---|---|---|---|---|---|
| CF | 1493 | 1328 | 1.58 | 1.30 | 122 | 209 | 8.61 | 8.43 |
| CB | 1184 | 1090 | 1.40 | 1.53 | 129 | 134 | 8.31 | 8.23 |
| MF | 1898 | 1086 | 2.02 | 1.63 | 87 | 143 | 8.51 | 8.44 |
| MB | 1983 | 1143 | 2.53 | 1.66 | 189 | 151 | 8.28 | 8.30 |
| GF | 1453 | 986 | 1.69 | 1.91 | 109 | 129 | 8.38 | 8.15 |
| GB | 624 | 981 | 0.99 | 1.98 | 179 | 139 | 8.30 | 8.32 |
| MGF | 835 | 1142 | 2.81 | 2.31 | 83 | 187 | 8.57 | 8.27 |
| MGB | 1179 | 1118 | 1.62 | 1.46 | 147 | 122 | 8.38 | 8.29 |

Treatment Key

| | |
|---|---|
| CF: Control Flat | CB: Control Bedded |
| MF: Mulch Flat | MB: Mulch Bedded |
| GF: Gypsum Flat | GB: Gypsum Bedded |
| MGF: Mulch/Gypsum Flat | MGB: Mulch/Gypsum Bedded |

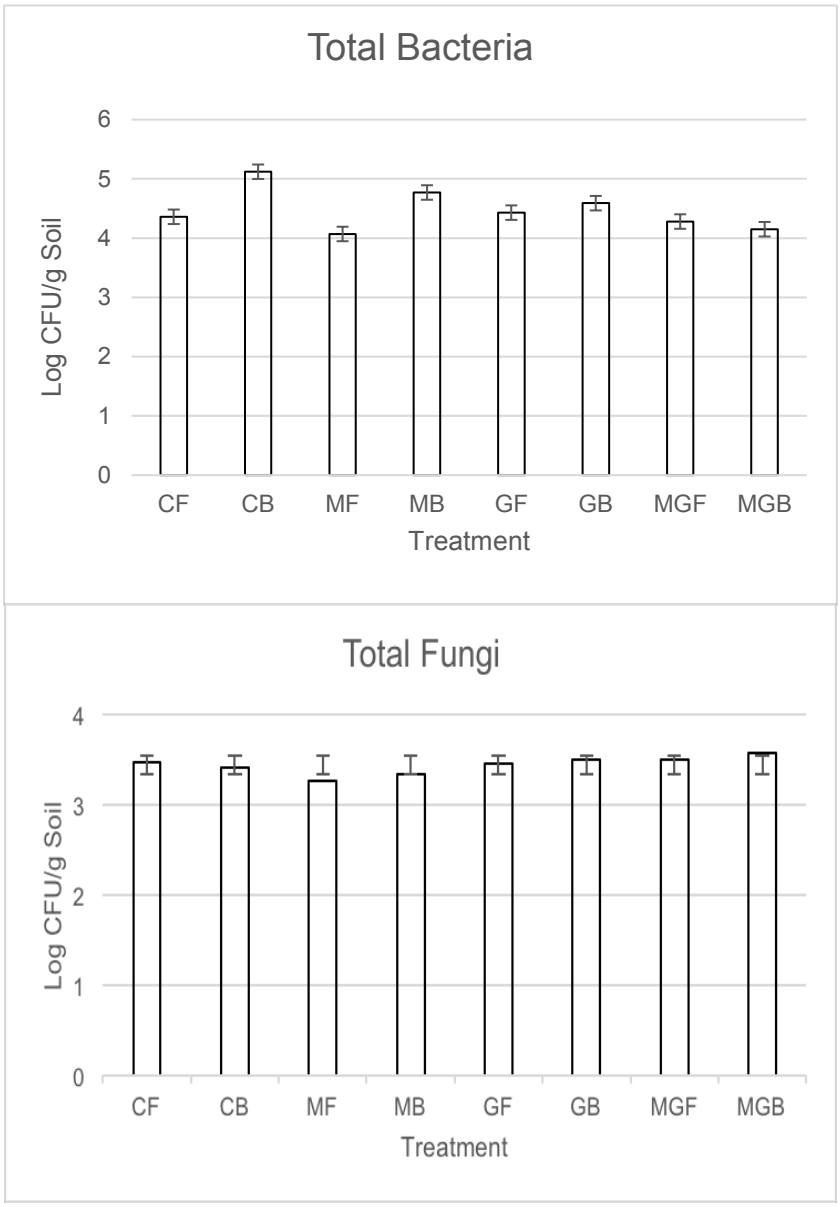
[1]Electrical Conductivity before amendments were applied, expressed in µS/cm
[2]Electrical Conductivity after amendments were applied
[3]Sodium Adsorption Ratio before amendments were applied
[4]Sodium Adsorption Ratio after amendments were applied
[5]Sodium concentration before amendments were applied, expressed in parts per million (ppm)
[6]Sodium concentration after amendments were applied, expressed in ppm
[7]pH before amendments applied
[8]pH after amendments applied

## Microbial Population Enumerations

Total bacteria populations in soil samples collected on July 19, 2016 ranged from 4.07 $\log_{10}$ CFU (colony forming units) per gram of soil to 5.12 (Figure 3), pseudomonads from 4.09 to 5.21, actinomycetes from 4.14 to 5.22 (Figure 4), and total fungi from 3.27 to 3.58. Bacteria, pseudomonad, and actinomycete populations were highest in the control bedded plots and lowest in the mulch flat plots. Fungi populations were highest in the mulch/gypsum bedded plots and lowest in the mulch flat plots (Table 3).

After conducting an analysis of variance (Table 4) there were no significant differences found between the treatments for fungi but bacteria (Table 5) and actinomycetes (Table 6) showed significantly higher populations in the control bedded treatment and significantly lower populations in the mulch flat treatment. Bacteria also showed significantly lower populations in the mulch gypsum bedded treatment plots. An analysis of variance was conducted on the soil moisture measurements (data not shown) to determine if moisture accounted for any of the differences detected, but there were no statistical differences in soil moisture between the treatments.

Total bacteria numbers were similar to pseudomonad and actinomycete populations indicating that the selective media used for pseudomonads and actinomycetes may not have been entirely effective. There were fluorescent

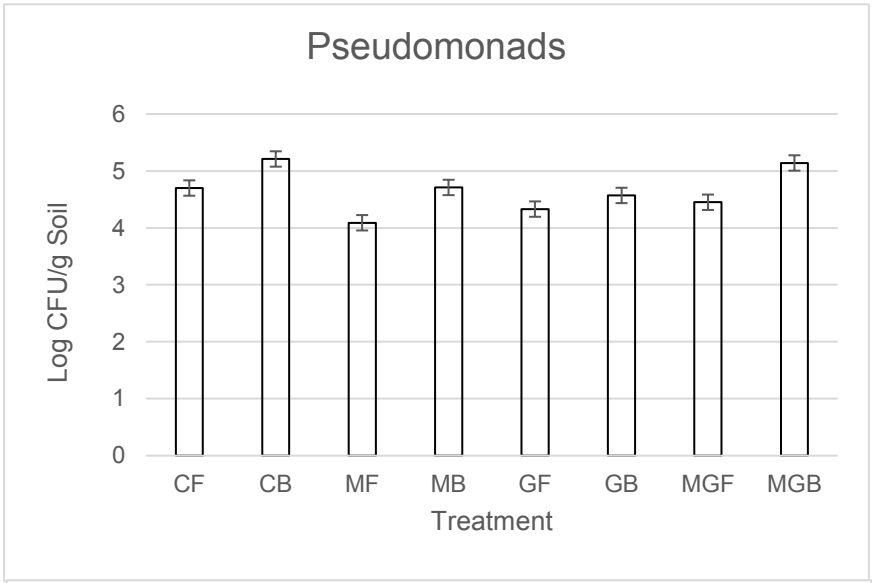


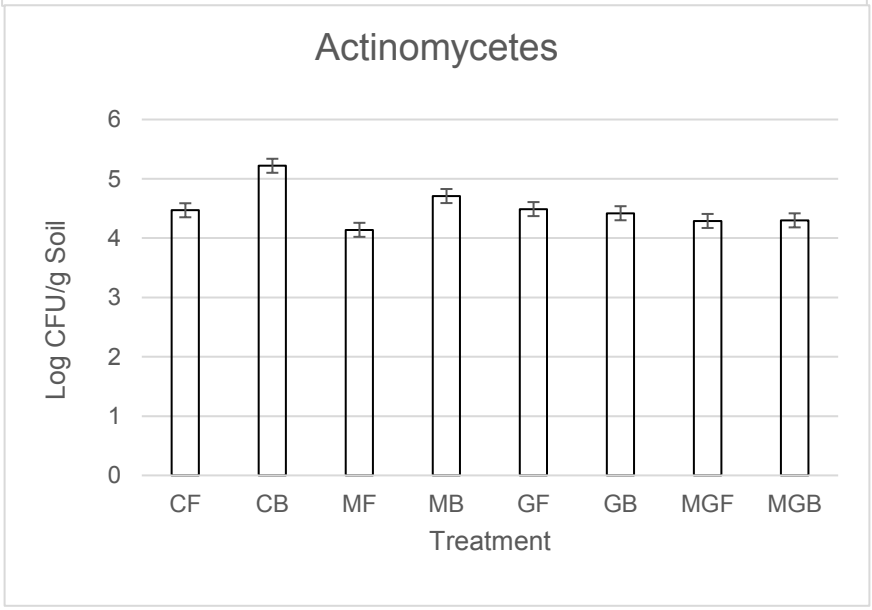

Error bars represent standard deviation within each treatment.

Treatment Key

| | |
|---|---|
| CF: Control Flat | CB: Control Bedded |
| MF: Mulch Flat | MB: Mulch Bedded |
| GF: Gypsum Flat | GB: Gypsum Bedded |
| MGF: Mulch/Gypsum Flat | MGB: Mulch/Gypsum Bedded |

Figure 3. Effects of Soil Treatments on Total Bacteria and Fungi Populations in Bulk Soil Samples Collected in July 2016.




Error bars represent standard deviation within each treatment.

Treatment Key

| | |
|---|---|
| CF: Control Flat | CB: Control Bedded |
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Figure 4. Effects of Soil Treatments on Total Pseudomonad and Actinomycete Populations in Bulk Soil Samples Collected in July 2016.

Table 3. Effect of Soil Treatments on Microbial Populations in Bulk Soil Samples Collected in July 2016.

| Treatment | Total Bacteria[1] | Total Pseudomonads | Total Actinomycetes | Total Fungi |
|---|---|---|---|---|
| CF | 4.36 | 4.70 | 4.47 | 3.48 |
| CB | 5.12 | 5.21 | 5.22 | 3.41 |
| MF | 4.07 | 4.09 | 4.14 | 3.27 |
| MB | 4.77 | 4.71 | 4.71 | 3.30 |
| GF | 4.43 | 4.33 | 4.49 | 3.46 |
| GB | 4.59 | 4.57 | 4.42 | 3.50 |
| MGF | 4.28 | 4.45 | 4.29 | 3.51 |
| MGB | 4.15 | 5.14 | 4.30 | 3.58 |

Treatment Key

| | |
|---|---|
| CF: Control Flat | CB: Control Bedded |
| MF: Mulch Flat | MB: Mulch Bedded |
| GF: Gypsum Flat | GB: Gypsum Bedded |
| MGF: Mulch/Gypsum Flat | MGB: Mulch/Gypsum Bedded |

[1]Populations expressed in $Log_{10}$ CFU (colony forming units) per gram soil

Table 4. Analysis of Variance of Microbial Populations in Bulk Soil Samples Collected in July 2016.

| Class | Effect | DF Effect | MS Effect | DF Error | MS Error | F | p-level |
|---|---|---|---|---|---|---|---|
| Total Bacteria | 1 | 7 | 0.4789 | 24 | 0.1747 | 2.74 | 0.0306 |
| Total Pseudomonads | 1 | 7 | 0.5860 | 24 | 0.1414 | 4.14 | 0.0041 |
| Total Actinomycetes | 1 | 7 | 0.4426 | 24 | 0.2058 | 2.15 | 0.0767 |
| Total Fungi | 1 | 7 | 0.0391 | 24 | 0.0326 | 1.20 | 0.3409 |

(α=0.05)

Table 5. Student Newman-Keul's Test Groupings for Bacteria Populations from Bulk Soil Samples Collected in July 2016.

| Treatment | Population Means[1] | SNK Grouping[2] |
|---|---|---|
| Control Bedded | 5.12 | A |
| Mulch Bedded | 4.77 | B, A |
| Gypsum Bedded | 4.59 | B, A |
| Gypsum Flat | 4.43 | B, A |
| Control Flat | 4.38 | B, A |
| Mulch Gypsum Flat | 4.28 | B, A |
| Mulch Gypsum Bedded | 4.15 | B |
| Mulch Flat | 4.07 | B |

[1]Populations expressed in $Log_{10}$ CFU (colony forming units) per gram soil
[2]Means with the same letter are not statistically different

Table 6. Student Newman-Keul's Test Groupings for Actinomycete Populations from Bulk Soil Samples Collected in July 2016.

| Treatment | Population Means[1] | SNK Grouping[2] |
|---|---|---|
| Control Bedded | 5.22 | A |
| Mulch Bedded | 4.71 | B, A |
| Gypsum Bedded | 4.49 | B, A |
| Control Flat | 4.47 | B, A |
| Gypsum Flat | 4.42 | B, A |
| Mulch Gypsum Bedded | 4.30 | B, A |
| Mulch Gypsum Flat | 4.29 | B, A |
| Mulch Flat | 4.14 | B |

[1]Populations expressed in $Log_{10}$ CFU (colony forming units) per gram soil
[2]Means with the same letter are not statistically different

colonies on some of the actinomycete plates, for example, a morphological characteristic usually indicative of the *Pseudomonas* genus, not an actinomycete.

Consistent with most microbial community population studies, total bacteria, including pseudomonads and actinomycetes outnumbered fungi, regardless of treatment or salt stress in the soil. In a study of forest soils for example, total bacteria ranged from 1.71 log CFU per gram soil to 2.39 while total fungi populations only ranged from 1.69 to 1.88 (Vieira and Nahas, 2005). A study of rhizosphere soil similarly showed total bacteria populations ranging from 4.8 to 6.3 log CFU per gram soil while total fungi populations were only 4.6 to 5.4 (Allen and Wagner, 2000).

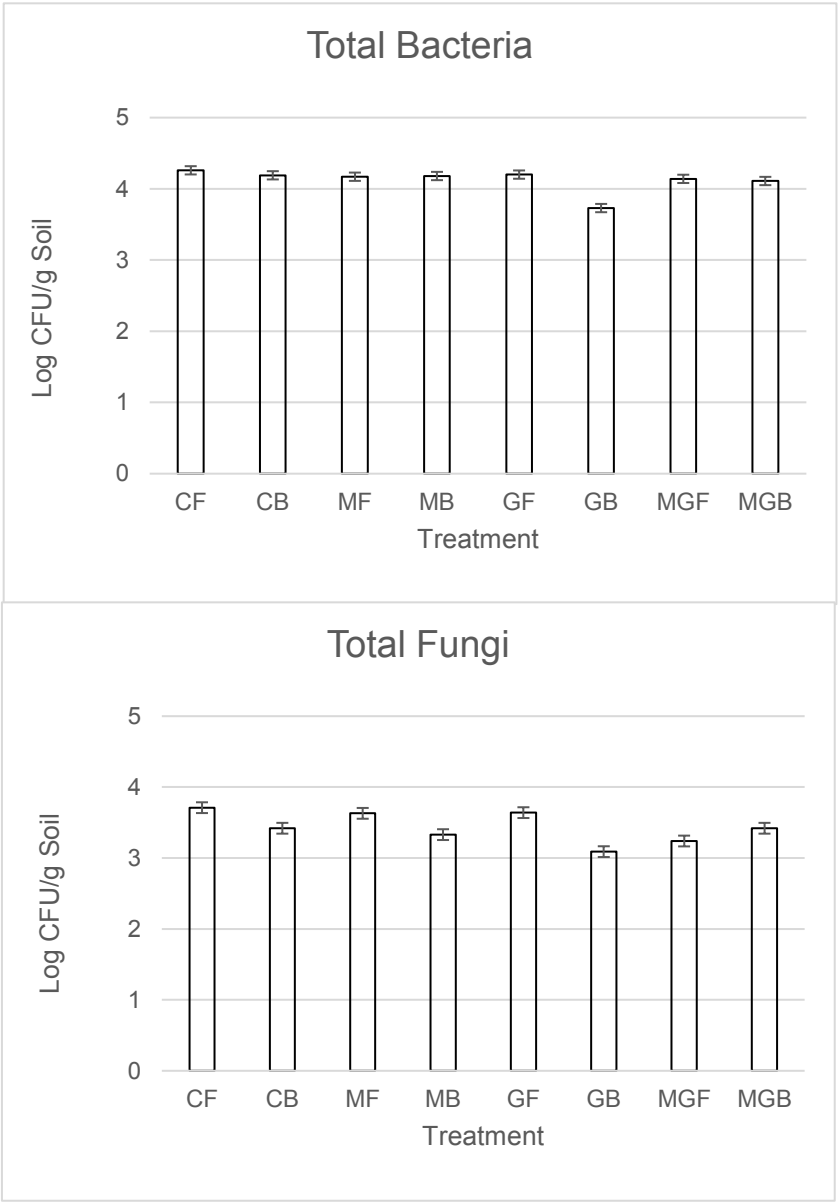
Previous studies saw success with stimulating microbial populations or mitigating their loss in coastal soils by using organic matter amendments. Sall et al., 2015 tested the effects of different salt concentrations on microbial biomass in soils amended with inorganic fertilizer and local organic matter and found the microbial biomass reduced by 50% in the control soil but only by 43% in the soil amended with organic matter at the highest salt concentration tested. This is the opposite of what was observed in the first samples, which had significantly lower bacteria, pseudomonad, and actinomycete populations in the plots amended with organic matter (pine bark mulch).

There were several significant rain events in the summer months, and the plots were well irrigated (drip and emitter systems). The influx of surface water that possibly pooled in the flat plots and the high availability of organic matter

stimulated not only the plants to extend new roots and photosynthesize, but also the microbial communities to decompose the organic matter, thus using much of the available oxygen. The high water content and low oxygen content could have accounted for the significantly lower microbial populations in the mulch flat treatment.

Most studies have found significant (30-50 percent) decreases in microbial biomass with increasing salinity (Elmajdoub et al., 2014). The microbial populations found in the tested soils were similar to those in soils impacted by a crude oil and brine spill from which salt tolerant organisms were also isolated (Allen and Wagner, 2000). Total bacteria at the crude oil and brine spill site ranged from 4.8 to 6.3 log CFU per gram soil while total fungi ranged from 4.6 to 5.4. Microbial populations impacted by an oil brine spill were also similar to those at the study site (Watson, 2006). This indicates that the salinity levels in the surface soil of the test plots were not high enough to have a significant negative impact on microbial populations, just as the sodium concentrations did not affect tree mortality in the companion study.

Bacteria populations for soil samples collected on October 22, 2016 ranged from 3.73 to 4.26 log CFU/gram (Figure 5), pseudomonads from 3.76 to 4.28 (Figure 6), actinomycetes from 3.87 to 4.32, and fungi from 3.09 to 3.71. Bacteria and pseudomonad populations were highest in the control flat plots as with the summer sampling period, but the actinomycete populations were highest in the gypsum flat plots. The fungi showed high populations in the control flat




Error bars represent standard deviation within each treatment.

Treatment Key

| | |
|---|---|
| CF: Control Flat | CB: Control Bedded |
| MF: Mulch Flat | MB: Mulch Bedded |
| GF: Gypsum Flat | GB: Gypsum Bedded |
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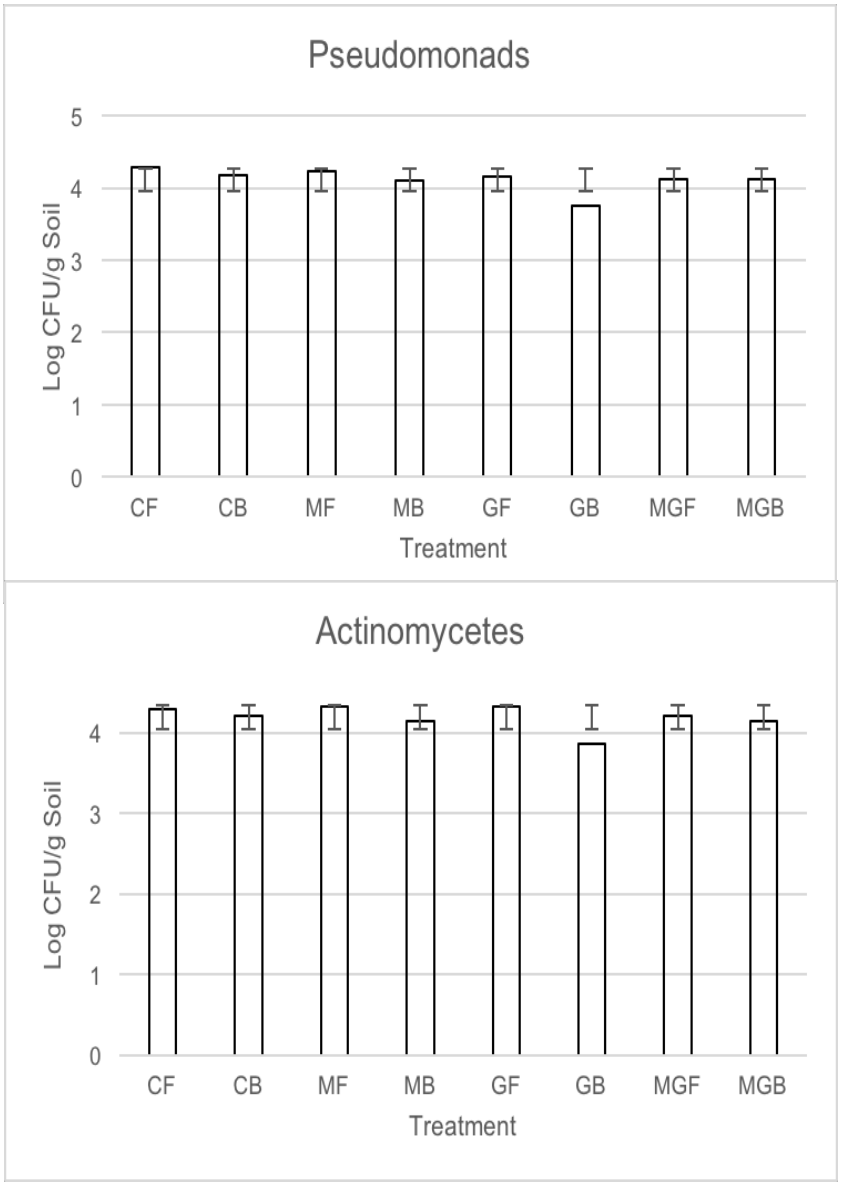
Figure 5. Effects of Soil Treatments on Total Bacteria and Fungi Populations in Bulk Soil Samples Collected in October 2016.




Error bars represent standard deviation within each treatment.

Treatment Key

| | |
|---|---|
| CF: Control Flat | CB: Control Bedded |
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Figure 6. Effects of Soil Treatments on Pseudomonad and Actinomycete Populations in Bulk Soil Samples Collected in October 2016.

plots, unlike in the summer sampling period. The gypsum bedded treatment showed the lowest populations for all four tested microbial communities (Table 7).

There were no statistical differences between the treatments for bacteria, pseudomonad, or actinomycete populations (Table 8). The fungi populations were highly variable with higher populations in the control flat treatment than in any other treatment, and a significantly lower population in the gypsum bedded treatment than in any other treatment (Table 9). The control, gypsum, and mulch flat treatments showed significantly higher populations than the other five treatments. The gypsum bedded and mulch gypsum flat populations were significantly lower than the other six treatment types.

The populations were all lower than in the summer sampling period, the decreased microbial activity possibly due to cooling temperatures. Populations in the fall sampling period were again similar to another study of coastal soils where spore forming bacteria populations ranged from 3.38 to 4.78 log CFU per gram soil (Azmi and Chatterjee, 2016). An analysis of variance was conducted on the soil moisture measurements to determine if moisture accounted for any of the differences detected, especially in total fungi, but there were no statistical differences in soil moisture among the treatments.

Consistent with the first sampling period, the fungi populations were still the lowest. The lack of consistent statistically significant differences in microbial

Table 7. Effect of Soil Treatments on Microbial Populations in Bulk Soil Samples Collected in October 2016.

| Treatment | Total Bacteria[1] | Total Pseudomonads | Total Actinomycetes | Total Fungi |
|---|---|---|---|---|
| CF | 4.26 | 4.28 | 4.29 | 3.71 |
| CB | 4.19 | 4.17 | 4.21 | 3.42 |
| MF | 4.17 | 4.23 | 4.32 | 3.63 |
| MB | 4.18 | 4.10 | 4.14 | 3.33 |
| GF | 4.20 | 4.15 | 4.32 | 3.64 |
| GB | 3.73 | 3.76 | 3.87 | 3.09 |
| MGF | 4.14 | 4.12 | 4.21 | 3.24 |
| MGB | 4.11 | 4.13 | 4.14 | 3.42 |

Treatment Key

| | |
|---|---|
| CF: Control Flat | CB: Control Bedded |
| MF: Mulch Flat | MB: Mulch Bedded |
| GF: Gypsum Flat | GB: Gypsum Bedded |
| MGF: Mulch/Gypsum Flat | MGB: Mulch/Gypsum Bedded |

[1] Populations expressed in $Log_{10}$ CFU (colony forming units) per gram of soil

Table 8. Analysis of Variance of Microbial Populations in Bulk Soil Samples Collected in October 2016.

| Class | Effect | DF Effect | MS Effect | DF Error | MS Error | F | p-level |
|---|---|---|---|---|---|---|---|
| Total Bacteria | 1 | 7 | 0.1010 | 24 | 0.0852 | 1.29 | 0.2972 |
| Total Pseudomonads | 1 | 7 | 0.6928 | 24 | 0.0532 | 1.86 | 0.1217 |
| Total Actinomycetes | 1 | 7 | 0.0856 | 24 | 0.0504 | 1.70 | 0.1572 |
| Total Fungi | 1 | 7 | 0.1858 | 24 | 0.0414 | 4.49 | 0.0026 |

(α=0.05)

Table 9. Student Newman-Keul's Test Groupings for Fungi Populations from Bulk Soil Samples Collected in October 2016.

| Treatment | Population Means[1] | SNK Grouping[2] |
|---|---|---|
| Control Flat | 3.71 | A |
| Gypsum Flat | 3.64 | B, A |
| Mulch Flat | 3.63 | B, A |
| Control Bedded | 3.42 | B, A, C |
| Mulch Gypsum Bedded | 3.42 | B, A, C |
| Mulch Bedded | 3.33 | B, A, C |
| Mulch Gypsum Bedded | 3.24 | B, C |
| Gypsum Bedded | 3.09 | C |

[1]Populations expressed in $Log_{10}$ CFU (colony forming units) per gram soil
[2]Means with the same letter are not statistically different

populations led to the discontinuation of a third full sampling period. Samples collected in January were only collected from control treatments and only plated on Pseudomonas Isolation Agar amended with different salt concentrations to attempt to isolate salt tolerant or halophilic organisms.

Fluorescent Pseudomonad counts in soil samples taken from control flat and bedded plots on January 16, 2017 ranged from 2.75 log CFU per gram soil to 4.17 (Table 10). The bedded treatment growing on zero percent salt agar had the highest counts of fluorescent pseudomonads, while the control flat treatment growing on five percent salt had the lowest fluorescent pseudomonad counts. There were no statistical differences in fluorescent pseudomonads (Table 11) between the control flat and bedded treatments, nor were there significant differences between any of the tested salt concentrations.

Culture dependent methods such as those used to determine microbial populations in this study exclude organisms that cannot be cultured in the lab such as mushrooms, obligate anaerobes, mycorrhizal fungi, and some fastidious chemoautotrophic organisms that are difficult to culture on commercial lab media or cannot be grown without their associated plant. More sophisticated profiling methods such as 16S ribosomal RNA fingerprinting can identify the organisms present in soil microbial communities even if the organisms cannot be cultured easily in the lab. These methods may be used to identify some of the salt tolerant isolates preserved from the January sampling period in the future, but were not used for the general population assessments. Culturing methods

Table 10. Fluorescent Pseudomonad Populations in Control Soils Collected in January 2017 (CF=Control Flat, CB=Control Bedded).

| Treatment | Fluorescent Pseudomonads[1] |
|---|---|
| CF (0% Salt) | 3.75 |
| CF (5% Salt) | 2.75 |
| CF (10% Salt) | 3.39 |
| CB (0% Salt) | 4.17 |
| CB (5% Salt) | 3.82 |
| CB (10% Salt) | 3.51 |

[1]Populations expressed in $Log_{10}$ CFU (colony forming units) per gram soil

Table 11. Analysis of Variance of Fluorescent Pseudomonad Populations in Control Soil Samples Collected in January 2017.

| Class | Effect | DF Effect | MS Effect | DF Error | MS Error | F | p-level |
|---|---|---|---|---|---|---|---|
| Treatment | 1 | 5 | 0.9297 | 18 | 0.7993 | 1.16 | 0.3649 |

(α=0.05)

assess general numbers of broad types of microbial communities using selective media and require little funding unlike population assessments that use 16S rRNA fingerprinting and identify specific genera present in the soil (Vasileiadis et al., 2012).

## Soil Respiration Determination

Respiration ranged from 0.873 grams of carbon dioxide per square meter of soil, per hour (g $CO_2$ $m^{-2}$ $hr^{-2}$) to 3.07 (Table 12). The total respiration was significantly higher in the mulch bedded treatments; consistent with other studies (Sall et al., 2015) suggesting that increased organic matter will stimulate microbial activity (Table 13). The mulch/gypsum bedded treatments showed significantly lower respiration values, however, indicating a possible negative effect of gypsum on the respiration activity (Table 14). The gypsum bedded respiration values were also lower, though not significantly so. However, plant roots, insects such as the active ant communities on the plots, and earthworms also contribute to total respiration and were not excluded from or corrected for in the respiration measurements taken in the field.

Respirations rates in a study on soil in coastal marshes ranged from 0.202 to 1.19 grams of carbon dioxide per square meter of soil per hour, overall lower than observed values (Wigand et al., n.d.). The treatments with high organic

Table 12. Effect of Soil Treatments on Total Soil Respiration as Measured at the Moody Gardens Test Plots in October 2016.

| Treatment | Average Respiration[1] | Average Soil Temp (˚C) |
|---|---|---|
| CF | 2.25 | 24.7 |
| CB | 1.63 | 24.2 |
| MF | 2.56 | 24.6 |
| MB | 3.07 | 25.6 |
| GF | 2.56 | 23.5 |
| GB | 1.84 | 23.0 |
| MGF | 2.13 | 24.5 |
| MGB | 0.87 | 21.9 |

Treatment Key

CF: Control Flat
CB: Control Bedded
MF: Mulch Flat
MB: Mulch Bedded
GF: Gypsum Flat
GB: Gypsum Bedded
MGF: Mulch/Gypsum Flat
MGB: Mulch/Gypsum Bedded

[1]Expressed in grams of $CO_2$ per square meter of soil per hour

Table 13. Analysis of Variance of Soil Respiration Measurements Collected on October 22, 2016.

| Class | CV | Effect | DF Effect | MS Effect | DF Error | MS Error | F | p-level |
|---|---|---|---|---|---|---|---|---|
| Respiration | 37% | 1 | 7 | 1.36 | 16 | 0.603 | 2.26 | 0.0843 |

(α=0.10)

Table 14. Student Newman-Keul's Test Groupings for Soil Respiration Measurements Taken at the Moody Gardens Study Site in October 2016.

| Treatment | Respiration Means[1] | SNK Grouping[2] |
|---|---|---|
| Mulch Bedded | 3.07 | A |
| Mulch Flat | 2.56 | B, A |
| Gypsum Flat | 2.56 | B, A |
| Control Flat | 2.25 | B, A |
| Mulch Gypsum Flat | 2.13 | B, A |
| Gypsum Bedded | 1.84 | B, A |
| Control Bedded | 1.63 | B, A |
| Mulch Gypsum Bedded | 0.87 | B |

[1]Populations expressed in $Log_{10}$ CFU (colony forming units) per gram soil
[2]Means with the same letter are not statistically different

matter and high respiration rates were also showing higher respiration values than another study where values ranged from 0.247 to 0.440 grams of carbon dioxide per square meter of soil per hour (Han et al., 2014).

## Characterization of Fluorescent Pseudomonads

Randomly chosen fluorescent isolates from each treatment and salt concentration were all determined to be gram negative, catalase positive, and oxidase positive rod shaped bacterium. Most were negative for glucose fermentation, preliminarily confirming the identities were of the *Pseudomonas* genus (Holt et al., 2000). Preserved samples were labeled with names starting with "MG-" indicating they had come from the Moody Gardens test plots, followed by the plot number, a letter for shorthand identification, and the salt concentration from which they had originally been isolated (Table 15).

Pseudomonads were chosen to isolate for future study as possible inoculants to stimulate plant growth because of their metabolic diversity, generally high salt tolerance, ability to degrade complex molecules, and their high potential for containing plant growth promoting genes. One study (Egamberdieva et al., 2008) identified a species of *Pseudomonas* capable of stimulating seedling germination by over 20% even at the highest salt concentration tested compared with uninoculated control seedlings. Past studies have also had success

Table 15. Characterization of salt tolerant isolates to preliminarily confirm identities as being within the *Pseudomonas* genus. ("+" indicates a positive result, "-" indicates a negative result).

| Isolate Code | Treatment[1] | % Salt | Gram Reaction | Catalase | Oxidase | Glucose Fermentation |
|---|---|---|---|---|---|---|
| MG-8A5 | CB | 5% | - | + | + | - |
| MG-8B10 | CB | 10% | - | + | + | - |
| MG-9C5 | CF | 5% | - | + | + | - |
| MG-9D10 | CF | 10% | - | + | + | - |
| MG-11E5 | CB | 5% | - | + | + | - |
| MG-11F10 | CB | 10% | - | + | + | - |
| MG-19G10 | CF | 10% | - | + | + | + |
| MG-19H5 | CF | 5% | - | + | + | - |
| MG-25I5 | CB | 5% | - | + | + | - |
| MG-25J10 | CB | 10% | - | + | + | - |
| MG-39K5 | CF | 5% | - | + | + | - |
| MG-39L10 | CF | 10% | - | + | + | + |
| MG-42M5 | CB | 5% | - | + | + | - |
| MG-42N10 | CB | 10% | - | + | + | - |
| MG-47O5 | CF | 5% | - | + | + | - |
| MG-47P10 | CF | 10% | - | + | + | + |

[1] CB= Control Bedded, CF= Control Flat

promoting plant growth and salt tolerance with inoculants from the *Bacillus* genus (Shi et al., 2012)(Orhan and Gulluce, 2015).

Another study (Salomon et al., 2014) showed no plant growth promotion or water loss inhibition for plants grown in saline soil inoculated with *Pseudomonas fluorescens*, but plants inoculated with *Bacillus licheniformis* exhibited both growth promotion and water loss inhibition. Similarly, another study (Damodaran and Jha, 2014) identified six isolates from rhizosphere soil that significantly promoted the growth of test plants in saline soil and found all to be of the *Bacillus* genus.

# CONCLUSIONS

Total bacteria and fungi populations are at appreciable levels and salt tolerant organisms were found in every sample collected in January. Several of these fluorescent salt tolerant organisms show promise as plant growth promoting organisms, possibly of the *Pseudomonas* genus, though this will need further confirmation. Organic matter amendments did not always have the predicted positive effects, for example in the July sampling period the untreated control samples had significantly higher bacteria populations compared to the treatments with incorporated pine bark mulch. Overall, however there was no discernible pattern of effects caused by the soil amelioration treatments on microbial populations. Future work will incorporate field and greenhouse tests comparing the efficacy of the fluorescent salt tolerant organisms as inoculants to stimulate plant growth in coastal soils. Confirming the identity of the isolates and testing for plant growth promoting genes using 16S rRNA profiling will be useful in determining their value as well.

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APPENDIX A

Plot Map

Towards Offatt's Bayou →

| Plot Number | Treatment | Plot Number | Treatment |
|---|---|---|---|
| 48 | MGB | 24 | MF |
| 47 | CF | 23 | GF |
| 46 | MB | 22 | CB |
| 45 | MGF | 21 | GB |
| 44 | MGB | 20 | MGF |
| 43 | GB | 19 | CF |
| 42 | CB | 18 | MB |
| 41 | GF | 17 | MF |
| 40 | CB | 16 | MGB |
| 39 | CF | 15 | GB |
| 38 | GF | 14 | MGF |
| 37 | MB | 13 | MF |
| 36 | GF | 12 | MF |
| 35 | MGF | 11 | CB |
| 34 | MB | 10 | MGB |
| 33 | GB | 9 | CF |
| 32 | MF | 8 | CB |
| 31 | GB | 7 | CF |
| 30 | MGF | 6 | GF |
| 29 | MB | 5 | MGB |
| 28 | MB | 4 | GB |
| 27 | CF | 3 | MGB |
| 26 | MF | 2 | MGF |
| 25 | CB | 1 | GF |

Treatment Key

CF: Control Flat
MF: Mulch Flat
GF: Gypsum Flat
MGF: Mulch/Gypsum Flat
CB: Control Bedded
MB: Mulch Bedded
GB: Gypsum Bedded
MGB: Mulch/Gypsum Bedded

Towards Moody Gardens ↓

# APPENDIX B

## Tryptic Soy Agar, Hardy Diagnostics

| Ingredient | Amount |
|---|---|
| Deionized Water | 1000 mL |
| Agar | 15.00 g |
| Casein Peptone | 15.00 g |
| Soy Peptone | 5.00 g |
| Sodium Chloride | 5.00 g |
| Cyclohexamide (AMRESCO, LLC)[1] | 0.25 g |

[1]25 mg/L solution, dissolved in acetone, added after autoclaving by sterile membrane filtration

# APPENDIX C

## Pseudomonas Isolation Agar, HiMedia Laboratories

| Ingredient | Amount |
|---|---|
| Deionized Water | 1000 mL |
| Peptone | 20.00 g |
| Potassium sulfate | 10.00 g |
| Magnesium chloride | 1.40 g |
| Agar | 15.00 g |

# APPENDIX D

## Actinomycete Agar, HiMedia Laboratories

| Ingredient | Amount |
|---|---|
| Deionized Water | 1000 mL |
| Sodium caseinate | 2.00 g |
| L-Aspargine | 0.10 g |
| Sodium propionate | 4.00 g |
| Dipotassium phosphate | 0.50 g |
| Magnesium sulfate | 0.10 g |
| Ferrous sulfate | 0.001 g |
| Agar | 15.00 g |
| Cyclohexamide[1] | 0.25 g |
| Glycerin[2] | 5 mL |

[1]see Appendix B
[2]liquid added before autoclaving

# APPENDIX E

## Rose Bengal Agar, HiMedia Laboratories

| Ingredient | Amount |
|---|---|
| Deionized Water | 1000 mL |
| Peptone | 5.00 g |
| Dextrose | 10.00 g |
| Monopotassium phosphate | 1.00 g |
| Magnesium sulfate | 0.50 g |
| Rose Bengal | 0.05 g |
| Agar | 15.00 g |
| Streptomycin[1] | 0.075 g |

[1]7.5 mg/L solution, dissolved in deionized water, added after autoclaving by sterile membrane filtration

# Vita

Elaine Fowler graduated with very high honors from Cabell Midland High School, Ona, West Virginia in May 2011. She graduated from the Honor's Program at Heidelberg University, Tiffin, Ohio, with a Bachelor of Science in Chemistry with minors in Biology and Vocal Performance in May 2015. She entered the graduate school of Stephen F. Austin State University the following fall to pursue a Master of Science degree in Environmental Science. She received the degree of Master of Science in Environmental Science on May 13, 2017.

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This thesis was typed by Elaine N. Fowler.